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Correlation between Body Mass Index and Gut Microbiota in Adults

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Gut microbiota has been proposed as a new environmental risk factor responsible for obesity. So, the aim of this study was to assess the gut microbiota profile, with special consideration to *Lactobacilli*, *E. coli* and *S. aureus* viable bacterial count (VBC) in (CFU/gm) and their association with body mass index (BMI) and obesity using quantitative stool culture. This study was conducted on 100 individuals; 74 with disturbed BMI who were divided into subgroups, with no other associated comorbidities and 26 healthy average weight volunteers. Fresh stool samples were collected and cultured directly on MacConkey, xylose lysine deoxycholate, Blood and Man-Rogozha Sharpe agar and quantitatively with serial dilutions (10^{-2} – 10^{-9}) in sterile normal saline. *S. aureus* was not isolated in any group, while, *Lactobacilli* and *E. coli* were isolated from all groups. *Lactobacilli* have a significant low VBC in obese and the highest VBC in the normal weight group showing a negative correlation with BMI. While, *E. coli* had a significantly high VBC in obese and the lowest in the underweight group showing a positive correlation with BMI. In conclusion, low *Lactobacilli* VBC and high *E. coli* VBC are significantly related to increased BMI and thus obesity.

Introduction

The rapidly growing prevalence of obesity among children, adolescents and adults and the associated metabolic disorders has become a global health problem (Xiao *et al.*, 2014).

Body mass index (BMI) is a good measure of obesity. Increased BMI is an established risk factor for many diseases; including, hypertension, ischemic heart disease, stroke, diabetes mellitus, respiratory disorders, and cancer of the large intestine, kidney,

endometrium, and postmenopausal breast. It is also related to a significantly increased mortality (Whitlock *et al.*, 2009).

Obesity is a complex condition influenced by many factors as Genetic, endocrinal and environmental factors (Xiao *et al.*, 2014). Gut microbiota has been proposed as a new environmental risk factor responsible for the weight gain and the altered energy metabolism that accompanies the obese state. The gut microbiota enables enzymatic

digestion of nondigestible polysaccharides producing absorbable monosaccharides; and it activates lipoprotein lipase on intestinal epithelium, which causes rapid absorption of glucose and fatty acids, contributing to the fat mass expansion and weight gain (*Musso et al.*, 2011). Many studies in both human subjects and experimental animals showed a significant association between the increase of some bacterial groups and obesity [(*Bäckhed et al.*, 2004); (*Ley et al.*, 2005); (*Ley et al.*, 2006); (*Turnbaugh et al.*, 2009) and (*Cani et al.*, 2008)]. Some studies found that *Lactobacillus* genus was the most predominant type of microbiota in stool of overweight and obese persons (*Armougom et al.*, 2006); (*Ignacio et al.*, 2016), while other studies linked overweight and obesity to Enterobacteriaceae, specifically *E. coli* (*Santacruz et al.*, 2010 and *Karlsson et al.*, 2011) and *S. aureus* (*Santacruz et al.*, 2010). Also, elevated proportion of *Firmicutes* (*Lactobacilli*) and reduced population of *Bacteroidetes* (*Bacteroides*) has been associated with obesity (*Angelakis et al.*, 2012). Moreover, gut microbiota composition at the species level even in the same genus is related to body weight and obesity as *Lactobacillus reuteri* is found more frequently in obese subjects whereas, *Lactobacillus paracasei* and *Lactobacillus plantarum* are significantly associated with lean status (*Million et al.*, 2012). Although these faecal microflora variability at the species level is detected by molecular tools based on 16s rDNA sequence similarities such as fluorescent in-situ hybridization (FISH) and denaturing gradient gel electrophoresis (DGGE) (*Sharma et al.*, 2012). However, these methods are costly, tedious, and measure even non-viable organisms (*Sieuwerts et al.*, 2008).

So, the aim of this study was to assess the gut microbiota profile, with special consideration to *Lactobacilli*, *E. coli* and *S. aureus* viable

bacterial count (VBC) in (CFU/gm) and their association with body mass index (BMI) and obesity using quantitative stool culture.

Materials and Methods

This study was conducted on 100 adults; 74 with disturbed BMI with no other associated co morbidities who were selected randomly from obesity outpatient Clinic of Ain Shams University Hospitals and 26 healthy average weight volunteers were included as control group matched for age and gender over a study period from September 2013 to February 2014. Confidentiality of information was maintained and consent was taken from each participant.

Exclusion criteria for the included subjects (*Million et al.*, 2013)

Adults less than 18 years old, history of cancer colon, presence of inflammatory bowel disease, an acute or chronic diarrhea in the previous 4 weeks before taking the stool sample, antibiotic, prebiotic and probiotic administration of a period less than one month before faecal sampling, familial obesity, patients with associated co-morbidities as (Diabetes mellitus, hypertension, cardiovascular stroke, chronic liver disease, and renal diseases), patients with dyslipidaemia, hormonal or endocrinal disturbance as (Thyroid dysfunction, Cushing syndrome, Growth hormone insufficiency).

Clinical history was taken and laboratory investigations were done to exclude the associated co-morbidities. Anthropometric measurements [weight, height, waist circumference (WC), hip circumference (HC) and waist to hip ratio (WC/HC)] using measuring tape and scale were assessed.

The studied groups were divided according to *Million et al.*, (2013) into subgroups

according to BMI that was defined as weight/(height)² as follows:

(1) Obese group: (29) patients who were subdivided into

- Morbidly obese (50 > BMI > 40)
Six patients; their age ranged from 30-40 with mean 36.000 + 3.950.
- Obese group (40 > BMI > 30)
Twenty-three patients; their age ranged from 19-53 with mean 36.522 + 11.016.

(2) Overweight group: (23) patients

Twenty-three patients (30 > BMI > 25); their age ranged from 19-47 with mean 33.522 + 8.409.

(3) Average weight or lean group (control group): (26) patients

Twenty-six patients (25 > BMI > 19), their age ranged from 19-54 with mean 31.192 + 9.148.

(4) Underweight group (22) patients

Twenty-two patients (BMI < 19), their age ranged from 19-45 with mean 29.000 + 6.726.

Stool sample collection and processing

Fresh stool samples were collected in sterile screw capped containers and delivered to The Central Microbiology Laboratory of Ain Shams University Hospitals for qualitative and quantitative culture.

Qualitative and quantitative culture for viable bacterial count (VBC) was done according to *Sharma et al.*, (2012).

Qualitative culture: Part of the stool were cultured directly on MacConkey agar media

plates (Oxoid[®], UK); to identify the microbial growth pattern of gram negative bacteria for each patient, on Xylose lysine deoxycholate (XLD) agar media plates (Oxoid[®], UK) to exclude *Shigella* and carrier state of *Salmonella*, on Blood agar plates (Oxoid[®], UK) for isolation of gram positive bacteria, and on Man-Rogozha Sharpe agar media (MRs) (Oxoid[®], UK) for isolation of *Lactobacillus species*.

Quantitative culture for VBC (CFU/gm stool)

Approximately one gram of each stool sample was transferred to one mL sterile normal saline and mixed thoroughly. Stool samples were serially diluted (10⁻² – 10⁻⁹). Ten µL from each dilution was plated using the quadrant technique on Man-Rogozha Sharpe agar media (MRs) agar (Oxoid[®], UK) for isolation and enumeration of VBC of *Lactobacillus species*, incubated anaerobically at 37°C for 72-hours and on blood agar for isolation and enumeration of VBC of *S. aureus*, and *E. coli* which incubated aerobically for 48-hours at 37°C.

All colonies of different morphology grown on XLD, MacConkey and from the highest dilution of (MRs) and blood agar were identified using conventional biochemical methods. The viable bacterial count of *Lactobacilli*, *E. coli* and *S. aureus* was calculated from the highest dilution and expressed as CFU/gm.

Statistical analysis

Non-numerical data were expressed as numbers (%). Chi-square was used to compare between two variables in qualitative data. Linear Correlation coefficient was used for detection of correlation between two quantitative variables in one group. ANOVA test was used for comparison among different

times in the same group in quantitative data. Kruskal-Wallis test is a nonparametric equivalent to one-way ANOVA used to determine if there are statistically significant differences between two or more groups of an independent variable on a continuous or ordinal dependent variable. All the analyses were performed with commercially available software (SPSS version 17, SPSS, Inc., Chicago, IL, USA)

Results and Discussion

The demographics and Anthropometric measurements

The demographics and Anthropometric measurements of the studied groups were shown in table 1.

In our study, the age and gender were not statistically different between all studied groups. A significant difference in height and WC/HC ratio was found ($P < 0.05$) among the studied groups; with the highest height in the underweight group (mean 1.682 ± 0.122), and the lowest height in the morbidly obese group (mean 1.578 ± 0.056). While, the lean group had the highest WC/HC ratio, (mean 0.848 ± 0.037) and the morbidly obese group had the lowest ratio, (mean 0.793 ± 0.053). Also, there was a highly significant difference among the studied groups regarding WC and HC ($P < 0.001$); being the lowest in the underweight group, (mean 71.000 ± 1.380 , 88.182 ± 4.216) respectively. While both circumferences were the highest in the morbidly obese group (mean 92.833 ± 3.430 , 117.333 ± 5.785) respectively.

Distribution of gut flora among the studied groups

In our study, *S. aureus* was not isolated in any of the studied groups. The most abundant genera in all groups were *E. coli* (100%), and

Lactobacilli (97%). The gut microbiota had the greatest genera diversity in the low BMI groups (normal and underweight); *E. coli* (100%), *Lactobacilli* spp. (97.91%), *Citrobacter* spp. (58.33%), *Enterobacter* spp. (16.66%), *Morganella* spp. (12.5%), *Acinetobacter* spp. (10.41%), and *Providencia* spp. (10.41%). The diversity decreased as the BMI increased (overweight, obese and morbid obese); *E. coli* (100%), *Lactobacilli* spp. (97.15%), *Citrobacter* spp. (57.69%), *Acinetobacter* spp. (9.62%), *Morganella* spp. (5.76%), and *Serratia* spp. (3.85%). The prevalence of *Citrobacter* spp. was significantly higher in morbidly obese group ($P < 0.05$) while, *Enterobacter* spp. were present only in low BMI groups (normal weight and underweight) ($P < 0.05$). *Acinetobacter* spp. prevalence was higher in obese and underweight groups, with no significant difference ($P > 0.05$). Although *Lactobacilli* and *E. coli* were isolated from all groups with higher prevalence in normal and overweight groups, however this result was statistically insignificant ($P > 0.05$) (Table 2). In agreement with our study *Chiu et al.*, (2014) found that bacterial communities in Taiwanese 45 normal stool samples ($BMI \leq 24$) had a greater genera richness than those in 36 case samples (with a $BMI \geq 27$). The supervised analysis showed that, the most abundant genera of bacteria in normal samples were *Bacteroides* (27.7%), *Prevotella* (19.4%), *Escherichia* (12%), *Phascolarctobacterium* (3.9%), and *Eubacterium* (3.5%). The most abundant genera of bacteria in case samples were *Bacteroides* (29%), *Prevotella* (21%), *Escherichia* (7.4%), *Megamonas* (5.1%), and *Phascolarcto bacterium* (3.8%). Also, they found that, *Acinetobacter* spp. was positively correlated with obesity. The genera of *Citrobacter*, *Tatumella*, and *Acinetobacter* exhibited significant differences in both presence and proportions between normal and case samples. Their findings may be resulted

from their categorization of the studied groups into case and control groups without any exclusion criteria, associated comorbidities or hormonal disturbance that may affect the diversity of gut microbiota. Moreover, anaerobes were isolated successfully in their study in contrast to this study which focused mainly on aerobes and facultative anaerobes. Also, they used sequencing of PCR products which may detect dead bacteria that do not express their metabolic effect (Sepp *et al.*, 2013). While, Xiao *et al.*, (2014) found a positive correlation between *Citrobacter* and BMI ≥ 28 Kg m⁻². Million *et al.*, (2013) who categorize their study subjects into four groups; group I: obese subjects (BMI > 30 Kg m⁻²), group II: overweight subjects (BMI > 25 and < 30 Kg m⁻²), group III: lean subjects (BMI > 19 and < 25 Kg m⁻²) and group IV: anorexic subjects (BMI < 19 Kg m⁻²) found that, the prevalence of *Lactobacillus* was higher in obese compared to lean group (p= 0.06) and a higher frequency of *Lactobacillus* in individuals with BMI > 25 Kg m⁻² vs BMI < 25 Kg m⁻² (p= 0.06), with a threefold increase in *L. reuteri* occurrence in obese patients compared with lean subjects (P= 0.01), and a threefold increase between individuals with BMI > 25 Kg m⁻² compared to individuals with BMI < 25 Kg m⁻² (P=0.001). Also, they found that, the prevalence of *E. coli* was lower in obese compared with lean group (p= 0.006) and the presence of *Bacteroidetes* was associated with the absence of obesity (OR = 0.51; P= 0.02) however anaerobes were not assessed in this study.

Viable bacterial count of *Lactobacilli* in the studied groups

In our study, *Lactobacilli* VBC showed significant difference among the studied groups (P=0.006), with the highest concentration found in the normal weight group with a median of (6.3x10⁹ CFU/gm)

and the lowest concentration was in the Obese (8x10⁸ CFU/gm) (Table 3). In contrast to our results, Armougom *et al.*, (2006) and Million *et al.*, (2012) found that *Lactobacillus* spp. concentration was significantly higher in obese subjects than lean control by quantitative PCR (qPCR). Also, Million *et al.*, (2013), found that, *Lactobacillus* concentration was higher in obese patients compared with lean patients (P<0.05) and in individuals with BMI > 25 Kg m⁻² vs individuals with BMI < 25 Kg m⁻². But Million *et al.*, (2012) found that, *Lactobacillus* spp. concentration by culture on *Lactobacillus* specific culture was not significantly different between obese and control subjects median (4.15 vs. 5.2log₁₀ CFUml⁻¹) respectively. However, our results could be explained by Million *et al.*, (2012) who found that, not only the concentration of *Lactobacilli* is the only discriminator between lean and obese individuals but also, the species did. They showed that *Lactobacillus paracasei* and *Lactobacillus plantarum* had a significant higher level in normal weight by culture, and qPCR, however, only *Lactobacillus paracasei* was significantly associated with lean status (odds ratio = 0.79; p= 0.03), while *Lactobacillus reuteri* had a significant higher level in obese subjects by qPCR not by culture. Also, Million *et al.*, (2013) showed that *Lactobacillus reuteri* concentration was positively correlated with BMI.

Viable bacterial count of *E. coli* in the studied groups

Also, in our study, the *E. coli* VBC was significantly different among different groups (P<0.001) being the highest in obese group (2.4x10¹⁰CFU/gm), and the lowest in the underweight group (4x10⁷CFU/gm) (Table 4). Similarly, Santacruz *et al.*, (2010) found that, *Enterobacteriaceae* (P=0.001), *E. coli* (P=0.005) and *Staphylococcus* (P=0.006)

numbers by qPCR were lower in normal-weight BMI < 25 Kg m⁻² than in overweight BMI >25 Kg m⁻² pregnant women. In contrast, *Million et al.*, (2013) found that, the concentration of *E. coli* was lower in obese compared with lean group (P=0.02), anorexic (P=0.001) and overweight individuals (P=0.012) and in individuals with BMI > 25 vs. < 25 Kg m⁻² showing that, a higher concentration of *E. coli* was associated with a lower BMI.

Correlation between anthropometric measures and VBC of both *Lactobacilli* and *E. coli*

There was a significant positive correlation between *Lactobacilli* VBC and height r=0.221, and a significant negative correlation between VBC of *Lactobacilli* and

BMIr=0.235 (Fig. 1). On the other hand there was a significant positive correlation between VBC of *E. coli* and BMI r=0.002 (Fig. 2), weight r=0.008, waist circumference r=0.002, hip circumference r=0.016. On the contrary to our study *Million and his co-workers (2013)* found that, higher concentration of *E. coli* was associated with lower BMI, while, higher concentration of *Lactobacillus reuteri* was associated with higher BMI.

To our knowledge, these results provide new insights into the correlation between gut microbiota and the rising trend in obesity in our population as this issue will be of great importance in the management of this growing worldwide problem and with special considering of the flourishing market of probiotics (*Million et al.*, 2012).

Table.1 The studied participants’ characteristics and demographics

Participant characteristics	Under weight BMI <19 (n=22) Mean ± 2 SD	Normal weight 25 > BMI >19 (n=26) Mean ± 2 SD	Over weight 30 > BMI >25 (n=23) Mean ± 2 SD	Obese 40 > BMI >30 (n=23) Mean ± 2 SD	Morbid obesity 50 > BMI > 40 (n=6) Mean ± 2 SD
Age (year)	19-45 (29.000±6.726)	19 – 54 (31.192±9.148)	19 - 47 (33.522±8.409)	19 – 53 (36.522±11.016)	30 – 40 (36.000±3.950)
Weight (kg)	38 – 62 (50.409±6.464)	49 -77 (59.365±8.219)	57-102 (79.478±13.36)	67 – 120 (88.348±12.662)	99 - 123 (109.5± 8.888)
Height (meters)	1.49 – 1.9 (1.682±0.122)	1.49 – 1.88 (1.628±0.104)	1.47 – 1.88 (1.680±0.128)	1.47 – 1.8 (1.593±0.087)	1.53 – 1.68 (1.578±0.056)
Waist circumference (cm)	68 – 74 (71.000±1.380)	70 – 90 (79.885±5.743)	77 – 102 (83.826±5.734)	80 – 98 (89.087±3.848)	87 – 97 (92.833±3.430)
Hip circumference (cm)	83 – 99 (88.182±4.216)	80 – 104 (94.192±5.987)	88 – 110 (99.435±5.106)	92 – 117 (106.870±7.015)	110–123 (117.333±5.785)
Waist circumference/ Hip circumference (cm)	0.714 – 0,881 (0.807±0.040)	0.777 – 0.917 (0.848±0.037)	0.709 – 1.000 (0.845±0.063)	0.714 – 0.922 (0.837±0.060)	0.719 – 0.864 (0.793±0.053)

Table.2 Distribution of fecal gut flora among the studied groups (n=100)

	Groups										Chi-Square	
	Under weight (n=22)		Normal weight (n=26)		Over weight (n=23)		Obese (n=23)		Morbid obesity (n=6)			
	N	%	N	%	N	%	N	%	N	%	X ²	P-value
<i>Citrobacter</i>	8	36.36	20	76.92	15	65.22	10	43.48	5	83.33	9.968	0.041*
<i>Enterobacter</i>	3	13.64	5	19.23	0	0.00	0	0.00	0	0.00	9.927	0.042*
<i>Morgenella</i>	2	9.09	4	15.38	3	13.04	0	0.00	0	0.00	4.622	0.328
<i>Serratia</i>	0	0.00	0	0.00	2	8.70	0	0.00	0	0.00	6.832	0.145
<i>Acinetobacter</i>	4	18.18	1	3.85	1	4.35	4	17.39	0	0.00	5.610	0.230
<i>Providencia</i>	3	13.64	2	7.69	0	0.00	0	0.00	0	0.00	6.588	0.159
<i>E.coli</i>	22	100.00	26	100.00	23	100.00	23	100.00	6	100.00	x	x
<i>Lactobacilli</i>	21	95.45	26	100.00	23	100.00	22	95.65	5	83.33	5.691	0.223

Table.3 Comparison between studied groups as regards fecal *Lactobacilli* VBC in (CFU/gm)

Groups	<i>Lactobacilli</i> VBC (CFU/gm)				Kruskal-Wallis Test	
	Range	Median	Interquartile Range	Mean Rank	X ²	P-value
Underweight	1.5×10 ⁸ -2. ×10 ¹⁰	2.8×10 ⁹	1.87×10 ¹⁰	56.21	14.461	0.006
Normal	6×10 ⁶ -7×10 ¹⁰	6.3×10 ⁹	3.51×10 ¹⁰	61.35		
Overweight	3.5×10 ⁶ -6.6×10 ¹⁰	2.4×10 ⁹	3.90×10 ⁹	45.35		
Obese	2×10 ⁷ -3.7×10 ¹⁰	8×10 ⁸	3.05×10 ⁹	36.66		
Morbid obesity	2×10 ⁷ -4×10 ⁹	2×10 ⁸	2.4×10 ⁹	25.60		

Table.4 Comparison between studied groups as regards faecal *E. coli* VBC in (CFU/gm)

Groups	<i>E. coli</i> VBC (CFU/gm)				Kruskal-Wallis Test	
	Range	Median	Interquartile Range	Mean Rank	X ²	P-value
Underweight	2×10 ⁷ -1×10 ¹⁰	4×10 ⁷	3.8×10 ⁸	18.09	43.067	<0.001*
Normal	5×10 ⁷ -1×10 ¹¹	3.95×10 ⁹	2.05×10 ¹⁰	51.02		
Overweight	6.3×10 ⁶ -8.4×10 ¹⁰	7.7×10 ⁹	9.50×10 ⁹	56.46		
Obese	2.4×10 ⁸ -1.33×10 ¹¹	2.4×10 ¹⁰	5.82×10 ¹⁰	73.43		
Morbid obesity	2×10 ⁸ -2.6×10 ¹⁰	9.5×10 ⁹	1.59×10 ¹⁰	56.33		

Fig.1 Correlation between faecal *Lactobacilli* (VBC) and (BMI)

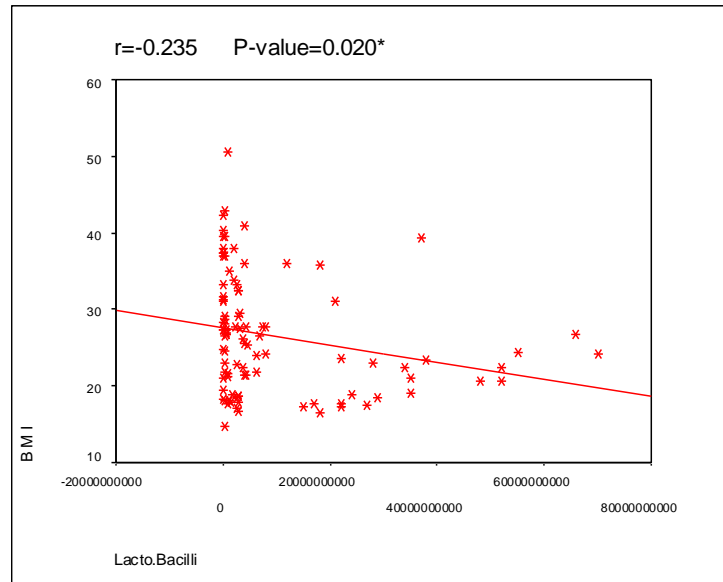
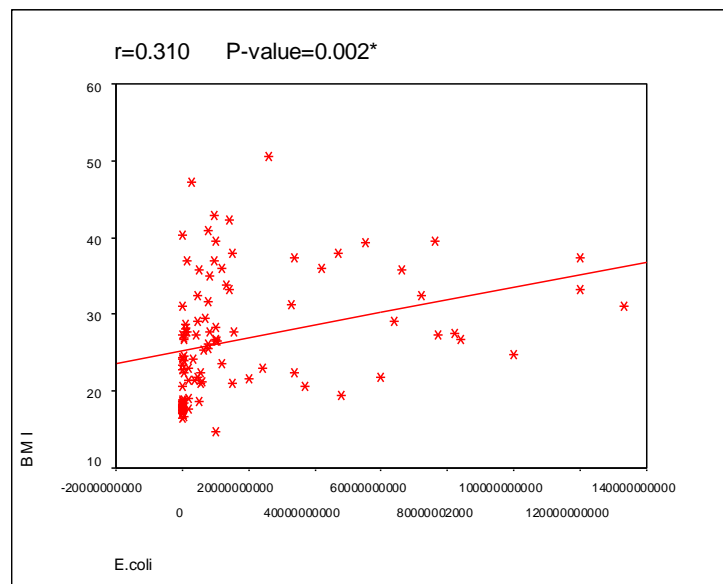


Fig.2 Correlation between faecal *E. coli* (VBC) and BMI



The limitation of this study was the extremes of BMI (morbid obesity and underweight individuals) which necessitates the increase of samples size in further studies, to avoid outliers. In addition, *S. aureus* couldn't be detected in this study despite its positive correlation with obesity as *Santacruz et al.*, (2010) found that the increase of *S. aureus* numbers were related to cases with increased plasma cholesterol levels which were

excluded in this study. Also, the anaerobic profile needs to be assessed and correlated to identify its role in disturbed body mass index.

In conclusion, Low *Lactobacilli* VBC and high *E. coli* VBC are significantly related to increased BMI and thus obesity, while, high *Lactobacilli* VBC is significantly related to average weight individuals.

This research recommends the identification of gut microbiota to the species level on a large scale of individuals to evaluate their distribution in obese persons and evaluate the significance of the use of certain strains of *Lactobacilli* as a probiotic as an adjuvant therapy for obesity.

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